44865709

Reviewer: S. Semalulu , Date April, 10, 2001

STUDY TYPE: Repeat-Dose Oral Toxicity [feeding-rat]; OPPTS 870.3100 (rodent), [§82-1]; OECD 408.

TEST MATERIAL (PURITY): SZX 0722 (99.4%) [Iprovalicarb]

SYNONYMS: Melody

CITATION: W. Bomann (1995): SZX 0722 - Subacute oral toxicity study in Wistar rats (28-day feeding study). Bayer AG, Report no. 24489 (November 20, 1995) unpublished.

SPONSOR: Bayer Corporation.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID not available), SZX 0722 technical (99.4 %) was administered to 10 SPF-bred Wistar rats (Bor: WISW[SPF-Cpb])/sex/dose, in the diet at dose levels of 0, 2000, 6000 and 20000 ppm (equal to 0, 195.8, 579.3, or 1973.9 and 0, 198.7, 572.8, or 1934.4 mg/kg bw/day in males and females respectively), for 28 days. There were no treatment related clinical signs, nor mortalities in any dose group. A slight reduction in food intake was observed in females at 20000 ppm in the first week but was later fully compensated and had no effect on body weight development. A slight reduction in body weight development occured in males at 20000 ppm in the first week, but subsequent body weight gain measurements of that group did not differ from controls. These transient small reductions in body weight gain were not considered biologically significant. There were significant and dose related increases in cholesterol and triglyceride levels and elevation of the absolute and relative liver weights of females at 6000 ppm and above, and increases in alkaline phosphatase activity, plasma cholesterol levels and relative (compared to body mass) liver weights of the males at 20000 ppm. The liver weight changes were considered toxicologically significant because of the associated cholesterol, triglyceride or alkaline phosphataes changes. There were no treatment related histological lesions in any of the tissues examined.

572.8 1973.9

The LOAEL in females was 6000 ppm, based increases in cholesterol and triglyceride levels and elevation of the absolute and relative liver weights at that dose. The NOAEL in females was 2000 ppm (195.8 mg/kg bw/d). The LOAEL in males was 20000 ppm, based on increases in alkaline phosphatase activity, plasma cholesterol levels and relative liver weights at that dose. The NOAEL in males was 6000 ppm (579.3 mg/kg bw/d)

This 28-day feeding study is classified satisfactory and satisfies the guideline requirement (OECD 407) for a repeat-dose oral study in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

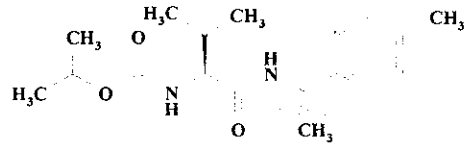
I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** SZX 0722

Description:	Technical, a white powder
Lot/Batch #:	NLL 4812-6.1
Purity:	99.4 % a.i.
Compound Stability:	Stable at room temperature
CAS #:	

Structure



2. **Vehicle and/or positive control:** 1 % DAB 9 Peanut (Batch # 904) to minimize dust formation.

3 **Test animals:**

Species:	Rat	
Strain:	Bor:WISW (SPF-Cpb)	
Age/weight at study initiation:	5-6 weeks/93-119 g, males; 88-109 g females.	
Source:	Winkelmann Experimental Animal Breeders, Brochen, Kreis Paderborn	
Housing:	Group caged by sex in Type III macrolon during acclimatization, and individually caged in Type II Macrolon cages during dosing.	
Diet:	Altomin rat maintainance diet, # 1324 (during acclimatization), *and* # 1321 during dosing (Altromin, GmbH, Lage), fed *ad libitum*	
Water:	Tap water in polycarbonate bottles, provided *ad libitum*	
Environmental conditions:	**Temperature:**	21 ± 1-5° C
	Humidity:	40-70%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/ 12 hrs artificial light
Acclimation period:	9 days	

B. STUDY DESIGN:

1. **In life dates** - Start:September 1992. End: October 1992)

2. **Animal assignment**:
Animals were assigned randomly to the test groups noted in Table 1 using a computer generated list of random numbers generated by the Randu computer program (IBM Scientific Subroutine Package).

TABLE 1: Study design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg bw)		# Male	# Female
		male	female		
Control	0	0	0	10	10
Low	2000	195.8	198.7	10	10
Mid	6000	579.3	572.8	10	10
High	20000	1973.9	1934.4	10	10

3. Diet preparation and analysis

Diets were prepared weekly by mixing appropriate amounts of test substance with the maintainance food and was stored at room temperature. Homogeneity of the diet mixture and stability were tested on feed samples collected. During the study, the first and last samples of treated food samples were collected and analysed for the concentration of the test material.

Results - Homogeneity Analysis: 97.0 - 107 % of nominal concentration

Stability Analysis: 98.6 - 107% of nominal concentration over a 14 day period

Concentration Analysis: 90.8 - 112% of nominal concentration

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics - Arithmetic group means and standard deviations were calculated from the individual animal results for body weight, food and water intake, organ weights, and hematological and clinical chemistry parameters. Other test results were compared between cohorts and controls using the rank tests (U tests) of Mann and Whitney, and Wilcoxon. Significant levels were set at $\alpha = 5\%$ ($p = \leq 0.05$) and $\alpha = 1\%$ ($p = \leq 0.01$). The statistical methods used are acceptable.

C. METHODS:

1. Observations:

Animals were inspected at least once every day for signs of toxicity and mortality.

2. Body weight:

Animals were weighed prior to study initiation, at the beginning of each study week thereafter and immediately prior to necropsy.

3. Food consumption and compound intake:

Food and water intakes for each individual animal were determined once weekly. Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency (body weight gain in kg/food consumption in kg per unit time X 100) and compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination:

Eyes of all animals were examined prior to study initiation. The control and high dose group were subjected to further opthalmological examination at study termination.

5. Haematology & Clinical Chemistry:

Blood was collected from 5 animals/dose group at the end of the treatment period from overnight fasted animals, for haematology and clinical analysis. For glucose analysis blood was collected from either the distal vessels following tail resection or by puncture of retro-orbital sinus. Blood for other purposes was drawn by heart puncture under diethyl ether anaesthesia. To determine liver microsomal enzyme activities, liver sections (0.9 - 1.2 grams) were collected from each animal at necropsy.
The parameters checked (x) in the tabel below were examined.

a. Haematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*	x	Reticulocyte count
x	Blood clotting measurements*		
x	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	**ELECTROLYTES**	X	**OTHER**
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)	x	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
x	Creatine phosphokinase		
x	Lactic acid dehydrogenase (LDH)		
x	S Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
x	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

C. Clinical chemistry of liver tisues involved assays of N-demethylase, O-demethylase and cytochrome P-450.

6. Urinalysis*

Urine was collected from fasted animals at the end of the treatment period. The CHECKED (X) parameters were examined.

X		X	
x	Appearance	x	Glucose
x	Volume	x	Ketones
x	Specific gravity	x	Bilirubin
x	pH	x	Blood
x	Sediment (microscopic)	x	Nitrate
x	Protein	x	Urobilinogen

* Not required for subchronic studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The organs marked (XX) in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta*	x	Brain*
x	Salivary glands*	x	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)^{T}
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen*	x	Eyes (optic n.)^{T}
x	Jejunum*	x	Thymus*		
x	Ileum*				GLANDULAR
x	Cecum*		UROGENITAL	x	Adrenal gland*
x	Colon*	x	Kidneys*+	x	Lacrimal gland^{T}
x	Rectum*	x	Urinary bladder*	x	Mammary gland^{T}
x	Liver*$^{+}$	x	Testes*$^{+}$	x	Parathyroids*$^{++}$
x	Gall bladder*	x	Epididymides	x	Thyroids*$^{++}$
x	Pancreas*	x	Prostate		
		x	Seminal vesicle		OTHER
	RESPIRATORY	x	Ovaries	x	Bone
x	Trachea*	x	Uterus*	x	Skeletal muscle
x	Lung*			x	Skin
x	Nose			x	All gross lesions and masses*
x	Pharynx				
x	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines^{+} Organ weight required in subchronic and chronic studies.

II. RESULTS

A. Observations :

1. Clinical signs of toxicity -
Threre were no treatment related clinical signs in any of the test groups throughout the study.

2. Mortality -
There was no mortality in any test groups throughout the study.

B. Body weight and weight gain:

During the first week, there was transient slight reduction in body weight in males at 20000 ppm. The overall body weight gain in all test groups of either sex were within 10% of the control (Table 2). Therefore there was no toxicologically effect on body weight development in either sex at all dose levels.

TABLE 2. Average body weights and body weight gains during 4 weeks of treatment

Dose rate (ppm)	Body Weights (g)			3	4	Total Weight Gain	
	Week -1	Week 1	Week 2	Week 4	Week 13	g	% of control
Male							
0	104	147	184	225	261	157	100
2000	103	143	179	219	251	148	94
6000	105	141	180	221	251	146	93
20000	101	136*	175	218	249	148	94
Female							
0	99	120	135	151	167	68	100
2000	96	119	135	146	162	66	97
6000	97	119	131	145	159	62	91
20000	96	116	131	144	158	62	91

[a] Data obtained from page (30) in the study report. * Significantly different ($p < 0.05$) from the control.
** Significantly different ($p < 0.01$) from the control.

C. Food consumption and compound intake:

1. Food and water consumption

A slight reduction in food intake occurred in the first week among females of the 20000 ppm dose group, but there was no appreciable effect on food intake at any other time point or in any other dose group. This transient change was no considered toxicologically significant. There was no treatment related effect on water consumption throughout the study.

2. Compound consumption

The calculated test compound intake (in mg/kg bw/day) is presented in Table1. The test compound consumed by the test groups corresponded to the theoretical differences in the dosage factors used.

3. Food efficiency There was no treatment related effect on food efficiency.

4. Ophthalmoscopic examination -

There were no treatment related opthalmological changes in any dose group throught the study.

E. Blood analyses

1. Haematology -

There was no evidence of treatment-related effects on the hematological parameters in all dose groups, throughout the study.

2. Clinical Chemistry -

Clinical chemistry data are presented in Table 3. In females, cholesterol and triglyceride levels were significantly elevated at 6000 ppm and above. This was considered toxicologically significant and treatment related. The enzyme induction assays showed that the O-demethylase activity and cytochrome P-450 mono-oxygenase system (P-450) were significantly increased in liver tissue of females at 6000 ppm and above. These changes were attributed to adaptive liver enzyme induction activity at that dose. Among males, there was a significant rise in the alkaline phosphatase activity and cholesterol level at 20000 ppm-group rats which were considered toxicologically significant treatment related effects. Creatinine and urea levels were decreased in the 6000 ppm and 20000 ppm-groups, but were not considered toxicologically significant. O-demethylase activity was statistically significantly increased at 6000 ppm and above and cytochrome P-450 levels were incresed at 20000 ppm, indicative of adaptive liver enzyme induction activity at that dose.

Table 3. Notable clinical chemistry changes in rats treated with SZX 0722 for 4 weeks

	0 ppm	2000 ppm	6000 ppm	20 000 ppm
Males				
ALP [U/l]	588	607	676	779**
CHOL [mmol/l]	2.38	2.38	2.44	2.88**
CREA [mmol/l]	57	53	47**	43**
UREA [mmol/l]	7.74	6.80	5.28**	6.30**
O-DEM [mU/g]	7.4	8.2	10.2**	15.4**
P-450 [nmol/g]	36.3	39.4	43.1	49.6**
Females				
CHOL [mmol/l]	2.27	2.34	2.78*	3.10**
TRIGL [mmol/l]	0.54	0.55	0.86*	0.99*
O-DEM [mU/g]	4.4	4.5	6.8**	9.5**
P-450 [nmol/g]	32.9	36.9	42.8*	44.0*

* U-test, 5 % significance level; ** U-test, 1 % significance level

F. Urinalysis -

There were no treatment related alterations in urine parameters.

G. Sacrifice and Pathology:

1. Organ weight -

Absolute and relative organ weights are presented in Table 4. In females, the absolute and relative liver weights were elevated at 6000 ppm and above. Among males, the relative liver weights were significantly elevated at 20000 ppm. These liver weight changes were considered treatment related as they had accompanying clinical chemistry changes. Other deviations of absolute or relative organ weights were not considered toxicological significant as they had no dose-response relationship, nor histological lesions or associated clinical chemistry changes.

Table 4: Organ weight changes in the 4-week feeding study with SZX 0722 in rats:

	0 ppm	2000 ppm	6000 ppm	20000 ppm
Females				
Absolute weight (mg)				
liver	6834	7207	7399*	7977**
pituitary	7	7	5*	6
Relative (organ/body) weight mg/100 g)				
liver	4092	4439	4637**	5062**
kidneys	734	775	769	788*
Males				
Absolute weight (mg)				
liver	11941	11396	11916	12591
pituitary	7	6	5*	5*
thyroid	5	5	6	6*
thymus	554	540	499	484*
Relative (organ/body) weight mg/100 g)				
pituitary	3	2	2*	2*
thyroid	2	2	2*	3*
liver	4561	4532	4713	5052**

+ U-test, 5 % significance level; ++ U-test, 1 % significance level

No histopathological lesions were detected in the examined organs and tissues.
Ophthalmoscopic examinations afforded no evidence for treatment related changes of the eyes.

2. Gross pathology -

There were no treatment related gross necropy findings at any dose level.

3. Microscopic pathology -

There were no treatment related histological findings at any dose level.

III. DISCUSSION

A. Investigators' conclusions:

The NOAEL in this study was 2000 ppm (equal to 195.8 and 198.7 mg/kg bw/day in males and females respectively) based on clinical chemistry findings (increase of cholesterol and triglyceride levels, decrease of creatinine and urea levels) elevation of 0-demethylase and cytochrome P-450 and elevated absolute liver weights at 6000 ppm and above.

B. Reviewer comments:

The induction of the O-demethylase activity and cytochrome P-450 mono-oxygenase system (P-450) in liver tissue in both sexes were attributable to adaptive liver enzyme induction activity at that dose, and were not necessarily an adverse effect. Likewise, the decrease in creatinine and urea levels were not deemed toxicologically significant.

The LOAEL in females was 6000 ppm (572.8 mg/kg bw/d), based on clinical chemistry changes (increase of cholesterol and triglyceride levels, associated with elevated absolute and relative liver weights) at that dose. The NOAEL in females was 2000 ppm (195.8 mg/kg bw/day).

The LOAEL in males was 20000 ppm (1973.9 mg/kg bw/d), based on increased cholesterol and triglyceride levels, and elevated relative liver weights at that dose. The NOAEL in males was 6000 ppm (579.3 mg/kg bw/day).

C. Study deficiencies: Thew were no major deficiencies in this study.